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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR No.: 0052014

MEMORANDUM

DATE: July 9, 2003

SUBJECT: Acifluorfen: Report of the Cancer Assessment Review Committee

FROM: Jessica Kidwell, Executive Secretary Jessica Kidwell
Cancer Assessment Review Committee
Health Effects Division (HED) (7509C)

TO: Byong-Han Chin, Toxicologist
and
Kit Farwell, Risk Assessor
Reregistration Branch 1
Health Effects Division (HED) (7509C)

Christina Scheltema, Chemical Review Manager
and
Mike Goodis, Branch Chief
Reregistration Branch 3
Special Review and Reregistration Division (7508C)

The Cancer Assessment Review Committee met on May 21, 2003 to re-evaluate the cancer classification of Acifluorfen. Attached please find the Final Cancer Assessment Document.

cc: R. Hill
J. Pletcher
Y. Woo

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CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF ACIFLUORFEN (THIRD REVIEW)
P.C. CODE: 114402

FINAL REPORT

JULY 9, 2003

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

2825

DATA PRESENTATION

Byong-Han Chin, Ph.D.

DOCUMENT PREPARATION:

Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE:

Karl Baetcke	
William Burnam	
Marion Copley	
Kit Farwell	
Abdallah Khasawinah	
Nancy McCarroll	
Tim McMahon	
Esther Rinde	
Jess Rowland	
Linda Taylor	Did not attend meeting

NON-COMMITTEE MEMBERS IN ATTENDANCE

John Pletcher, Consulting Pathologist	See attached sheet
Lori Brunsman, Statistical Analysis	

OTHER ATTENDEES:

Whang Phang (RRB1), Tim Dole (RRB1), Christina Scheltema (SRRD)

Nancy McCarroll _____

Tim McMahon _____

Esther Rinde _____

Jess Rowland _____

Linda Taylor _____

NON-COMMITTEE MEMBERS IN ATTENDANCE

John Pletcher, Consulting Pathologist _____

Lori Brunsman, Statistical Analysis _____

OTHER ATTENDEES:

Whang Phang (RRB1), Tim Dole (RRB1), Christina Scheltema (SRRD)

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EXECUTIVE SUMMARY

On May 21, 2003, the Cancer Assessment Review Committee (CARC) met to reevaluate the cancer classification of acifluorfen in light of the conclusions of the Mechanism of Toxicity Assessment Review Committee (MTARC) [TXR No. 0050227, May 14, 2003].

On April 17, 2003, the MTARC reviewed the merits of the toxicological data supporting peroxisome proliferation as the proposed mode of action for acifluorfen. Based on the available toxicity data, there is evidence to support that acifluorfen is a non-genotoxic hepatocarcinogen. The MTARC concluded that the currently available data are considered to be sufficient to support peroxisome proliferation as the mode of action of acifluorfen-induced liver tumors in mice according to the criteria recommended by International Life Sciences Institute (ILSI). The evidence includes the following:

1. Changes in liver morphology were observed in both rats and mice treated with acifluorfen. These effects include: dose-dependent increase in relative liver weights, and increased incidence of cellular hypertrophy, and increased number of peroxisomes.

2. Evidence of cell proliferation as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling in light microscopy.

3. Dose-dependent increase in the levels of CN-insensitive acyl (palmitoyl) CoA oxidase activities involved in peroxisomal fatty acid metabolism.

4. Previously, the MTARC concluded that peroxisome proliferation is the mode of action for lactofen in inducing liver tumors in rodents. Since acifluorfen is the major metabolite of lactofen in rodents, it is probable that acifluorfen contributes to peroxisome proliferation and liver tumors induced by lactofen. Therefore, the mode of action data on lactofen provide support for acifluorfen.

Based on the liver tumors seen in both sexes of the mouse, and in consideration of the mechanistic data provided, the CARC concluded that acifluorfen should be classified as "**likely to be carcinogenic to humans at high enough doses which cause the biochemical and histopathological changes in livers of rodents but unlikely to be carcinogenic at doses below those causing these changes.**" Furthermore, the CARC recommended using an MOE approach for estimating human cancer risk. A NOAEL of 25 ppm (1.25 mg/kg/day) from the chronic RfD was recommended to be used for calculating the margin of exposure (MOE).

The CARC also noted that the forestomach papillomas seen in both sexes of the mouse are of

questionable relevance to human health risk assessment since humans do not have a forestomach.

I. INTRODUCTION

On May 21, 2003, the Cancer Assessment Review Committee (CARC) met to evaluate the conclusions of the Mechanism of Toxicity Assessment Review Committee (MTARC) and to reevaluate the cancer classification of acifluorfen. The data were presented by Dr. Paul Chin of Reregistration Branch 1 of the Health Effects Division.

II. BACKGROUND INFORMATION

Acifluorfen produced liver tumors in two mouse carcinogenicity studies (MRID Nos. 00082897 and 00122732) employing different strains of mice (B6C3F1 and CR-CD-1) in both sexes. A two-year bioassay in the F-344 rat administering Tackle (approximately 20-24% acifluorfen) in the diet for 24 months failed to show any treatment-related increase in tumor incidence.

In 1987, the data were evaluated by the Carcinogenicity Peer Review Committee (CPRC) (HED Doc. No. 007698, September 30, 1987) and the Science Advisory Panel (SAP) (December 15, 1987). The CPRC concluded that acifluorfen should be classified as a B2 carcinogen based on liver tumors seen in mice. However, the SAP concluded that the weight of evidence for acifluorfen could be interpreted as supporting either a B2 or C classification.

In 1988, the CPRC reevaluated the carcinogenicity classification of acifluorfen (TXR. No. 007698, March 17, 1988) following the SAP review and concluded that the weight of the evidence on acifluorfen in mice was sufficient to categorize the chemical as a B2 carcinogen (probable human carcinogen) (HED Doc. No. 007698, March 17, 1988).

In 1999, the Hazard Identification Assessment Review Committee (HIARC) reviewed the data for acifluorfen and confirmed the decision reached by the CPRC in 1988. In 2001 a revised quantification was carried out using cross species scaling factor. The current Q_1^* is 1.27×10^{-2} $(mg/kg/day)^{-1}$ in human equivalents (TXR No. 0050263, November 8, 2001).

In 2001, the MTARC evaluated the proposed mechanistic data for lactofen of which acifluorfen is a major metabolite. The MTARC concluded that available data indicated that lactofen induced an increase in liver tumors through the induction of microsomal proliferation.

Subsequently, the registrant (BASF) submitted a petition (MRID No. 45323500) requesting that risk assessment for acifluorfen be based on the MOE approach rather than using a Q_1^* (from K. Blundell, BASF to Ms. Christina Scheltema, Chemical Review Manager, SRRD,

MRID No. 45323500 dated Feb. 2, 2001). The petition reviewed and summarized earlier data submissions, which provided the information to support the proposal of peroxisome proliferation as the mechanism of action of acifluorfen in inducing liver tumor in mice.

The data on the proposed mode of action submitted by the registrant were considered at a preliminary meeting of the Mechanism of Toxicity Assessment Review Committee (MTARC), HED on Aug. 23, 2001. The Committee evaluated the available data according to criteria recommended by the International Life Sciences Institute (ILSI) workshop on peroxisome proliferation for evaluating whether or not liver tumors were induced by acifluorfen via peroxisomal proliferation. The criteria are the following:

1. Changes in liver morphology indicating hepatomegaly as measured by increased relative liver weights and an increased number of peroxisomes as measured by morphometric analysis.

2. Evidence of cell proliferation as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling in light microscopy.

3. Increased levels of enzymes involved in peroxisomal fatty acid metabolism, especially CN-insensitive acyl (palmitoyl) CoA oxidase activities.

After thorough evaluation of the available data at that time, the MTARC concluded that the available information were insufficient to support this proposed mode of action of peroxisome proliferation for acifluorfen. BASF was informed of this decision (e-mail to R. Hawks, BASF, from B. H. Chin, EPA, dated 9/12/01). Subsequently the registrant proceeded to develop the necessary data and submitted the following new mechanism studies with acifluorfen:

1) The induction of the number and size of hepatic peroxisomes in mice following 4 week dietary feeding with acifluorfen (MRID 45693401),

2) S-phase response study in the liver of mice following 3 days, 1 week, and 2 weeks feeding with acifluorfen (MRID 45803601) and

3) Enzyme induction study in the liver of mice following 4 weeks feeding with acifluorfen (MRID 45793901).

On April 17, 2003, the MTARC reviewed all the available toxicological data supporting peroxisome proliferation as the proposed mode of action for acifluorfen. Based on the weight-of-evidence from currently available data, the MTARC concluded that the data provided sufficient support for the proposed mechanism of action for this chemical (TXR No. 0050227

dated May 14, 2003). The MTARC also recommended that the CARC should reevaluate the carcinogenicity classification of acifluorfen in light of the mode of action of acifluorfen as it pertains to the formation of liver tumors in mice.

III. STUDIES REVIEWED BY THE CARC

The CARC reviewed summaries of the mechanistic studies as presented in the report of the MTARC. These studies included several non-guideline *in vitro* and *in vivo* mechanism studies that characterized acifluorfen-induced peroxisome proliferation. Based on these data, the CARC noted that all peroxisome effects occurred at doses lower than doses where liver tumors were produced. In addition, the increased levels of enzymes (ALP and SGPT) seen at 40.5 mg/kg/day in the carcinogenicity study in mice (CD-1) were considered equivocal because of large standard deviations. Therefore, the first MTARC report was revised to reflect this conclusion (TXR No. 0052006, July 9, 2003).

Of the studies evaluated by the CARC, a non-guideline study (MRID 45803601), in which cell proliferation as measured in mouse livers by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling, was **considered most pertinent**. The increased cell proliferation (hepatocellular BrdU nuclear labeling) in comparison with two other events (increased size and number of peroxisome or palmitoyl CoA oxidase) was considered most pertinent because this is the **key event leading to liver tumor formation**. In this study (MRID 45803601), Blazer Technical (46.1% sodium acifluorfen in acetone) was administered to groups of 8 male and 8 female B6C3F1 mice at dietary concentrations of 0, 350, 1735 and 5210 ppm (*i.e.*: 160, 800 and 2400 ppm of the active ingredient), mean daily intakes of the a.i. for males/females of 40/54, 227/287 and 714/845 mg/kg/day, respectively, for 3 days, 1 week or 2 weeks. One week prior to necropsy, osmotic mini-pumps containing bromodeoxyuridine (BrdU) were implanted subcutaneously. Cell proliferation (S-phase response) and apoptosis were determined in the liver. This study showed that the oral administration of Blazer Technical (46.1% acifluorfen-sodium) to mice for 2 weeks produced a dose-dependent and significant induction of bromodeoxyuridine (BrdU) labeling in the liver, which is indicative of cell proliferation (S-phase response). The most pronounced effect for each of these three parameters (↑liver weight, ↑liver hypertrophy and ↑BrdU labeling) for both sexes was seen after 1 week of treatment.

Two other mechanism studies showed that oral administration of Blazer Technical (46.1% acifluorfen-sodium) to mice for 4 weeks induced a dose-related increase in the number, size, and area of hepatic peroxisomes in the liver (MRID 45693401) and also a dose-dependent increase in cyanide-insensitive palmitoyl-CoA-oxidation (PALCoA) (MRID 45793901).

Tables 1 and 2 show a "Summary of Peroxisomal Effects and Liver Tumor Induction in Male and Female Mice Administered Acifluorfen". **Tables 3 and 4** show a " Summary of Liver Toxicity and Tumor Induction in Male and Female Mice Administered Acifluorfen".

IV. DISCUSSION

After careful consideration of all the available data related to the liver tumor formation, the CARC concluded that there was support for the proposed mechanism of action for acifluorfen. The CARC also considered an appropriate endpoint or point of departure (POD) to be used as the hazard basis for the margin of exposure (MOE) calculations for cancer risk assessments.

The CARC decided that the hepatic cell proliferation study (MRID 45803601) in male mice was the best study to use for the POD because it provided very robust dose response data on an effect causally related to tumor formation. Acifluorofen induced a higher incidence of liver tumors in male mice than in female mice. Thus, the male mice cell proliferation data were used to estimate the POD. Cell proliferation (labeling indices) data at the 1-week interval were considered the most appropriate data to use to estimate the POD because PPAR (Peroxisome Proliferator Activating Receptor) agonists, such as acifluorfen, typically induce the maximum increase in cell proliferation during the first week of treatment. They provided the most suitable dose-response relationship. The lack of a NOAEL at the lowest dose of 40 mg/kg/day was addressed by using benchmark dose (BMD) methodology (USEPA, 2000). The CARC considered that only that dose which caused at least a doubling of cell proliferation was biologically meaningful (BMD_{2x}). Preliminary BMD analyses of male and female data from the three liver lobule zones and the combined data (all zones) indicated that combined data from male mice (all zones) resulted in the most protective estimate. The BMD analysis was performed using the Hill model (EPA Benchmark Dose Software, Version 1.3.1). Inspection of the curve and comparison of estimated and actual data indicated good fit (Appendix 1). For the combined data, the lower 95% confidence limit (i.e, $BMDL_{2x}$) on the calculated BMD_{2x} of 11.8 mg/kg/day was estimated to be 6.6 mg/kg/day.

The CARC, however, recommended that, instead of the dose of 6.6 mg/kg/day, the dose of 1.25 mg/kg/day that is currently used for all long-term or chronic risk assessments should be used for the cancer MOE calculations because it is protective of all endpoints. This value would also eliminate some of the uncertainty regarding the use of the BMD_{2x}. The 1.25 mg/kg/kg was derived from a 2-generation reproduction study in rats (MRID 00155548) with effects at 25 mg/kg based on kidney lesions in females of both generations. The CARC also recommended that the **MOE calculations** for cancer only be carried out in cases of chronic or long-term exposure and not where exposures were considered to be of short- or intermediate-term duration.

Chronic Toxicological Effects: Based on the results of a chronic toxicity/carcinogenicity in rats, the NOAEL for systemic toxicity is 500 ppm (25 mg/kg/day); the LOAEL is 2500 ppm (125 mg/kg/day) based on reduced body weight, increased absolute and relative liver weights and increased kidney weights, increased incidence of nephritis/pyelonephritis, increased incidence of acidophilic cells in the liver, and related changes in clinical chemistry parameters.

No treatment-related increase in tumor incidence was found in the acifluorfen treated rats.

Reproductive Toxicity: Based on the results of a two-generation reproduction study in the rat, the NOAELs for parental and offspring toxicity were established at 25 ppm (1.25 mg/kg/day). Effects observed at the LOAEL of 500 ppm (25 mg/kg/day) included parental toxicity consisting of kidney lesions, characterized predominantly by dilatation of tubules in the outer medulla, in females of both generations and offspring toxicity consisting of decreased viability and increased incidence of kidney lesions, characterized predominantly by dilatation of pelvis in F2 generation. In this study, the NOAEL for reproductive toxicity is equal to or greater than 2500 ppm (125 mg/kg/day) the highest dose tested and the LOAEL is not established.

Based on the results of this study, the chronic RfD was established at 0.013 mg/kg/day (NOAEL of 1.25 mg/kg/day and uncertainty factor of 100).

Other Carcinogenicity Issues: Based on the results of a carcinogenicity study in B6C3F1 mice, in addition to the liver tumors, the forestomach tumors (papillomas) were significantly increased above the controls at the highest dose level tested (2500 ppm) in both sexes. However, the CARC concluded that the forestomach papillomas are **of questionable relevance** to human risk assessment based on the fact that humans do not have a forestomach and the tumors appear to be related to the irritation potential of the pesticide. The following points were considered in deriving this conclusions:

1) The rodent stomach consists of the forestomach, the nonglandular portion (proximal to the esophagus) and the glandular portion (comprised of a fundus and pylorus). However, the entire human stomach consists of the glandular portion only and thus the forestomach papillomas are not a concern for humans.

2) The main function of the forestomach of the rodents "appears to be the storage of food prior to its entry into the glandular stomach which is responsible for the initial digestion of the food " and, therefore, the forestomach of the rodents was exposed to xenobiotics in the ingested food for longer duration (Clayson, *et al.*, 1990). Acifluorfen is category I and II for the eyes and skin irritation studies and thus the potential irritation following oral exposure may be the cause of the forestomach papillomas.

V. CANCER CLASSIFICATION

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July 1999), based on the liver tumors seen in both sexes of the mouse, and in consideration of the mechanistic data provided, the CARC concluded that acifluorfen should be classified as "**likely to be carcinogenic to humans at high enough doses which cause the biochemical and histopathological changes in the liver of rodents, but unlikely to be carcinogenic to humans below those doses causing these changes.**" Furthermore, the CARC recommended using an MOE approach for estimating human cancer risk. A NOAEL of 25 ppm (1.25 mg/kg/day) was recommended to be used for calculating the margin of exposure (MOE).

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Table 1. Summary of Peroxisomal Effects and Liver Tumor Induction in Male Mice Administered Acifluorfen

Dose (mg/kg/day) as active ingredient	Mean Number of Peroxisome according to size classification (relative to controls, %) MRID 45693401 (a)				Induction of Peroxisomal Enzyme Activities; Palmitoyl CoA oxidase MRID 45793901 (b)	Cell proliferation; BrdU labeling in the liver (S-phase response) MRID 45803601 (c)	Tumor incidence (% incidence) 18-month carcinogenicity study in mice **MRID 00122732** (d) (Note 2)		
	1 (<0.1 μm^2)	2 (<0.3 μm^2)	3 (<0.5 μm^2)	Total area of peroxisomes (note 1)			Adenomas	Carcinomas	Adenomas/ Carcinomas Combined
0	7.8 ±1.5 (SD)	8.0 ±2.0	0.5 ±0.4	1.25 ±0.18	5.24 ±0.5	1.01 ±0.3	8/58 (14 %)	1/48 (2 %)	9/58 (16 %)
37-42	**11.8* (51%) ±4.1**	9.6 (20%) ±1.7	0.3 ±0.2	1.49 (19%) ±0.29	**8.29** (58%) ±0.57**	**4.07 **(304%) ±1.86**	NT	NT	NT
119	NT	NT	NT	NT	NT	NT	**18/60 (30 %) ***	3/50 (6 %)	**21/60 (35 %) ***
180-227	**15.4** (97%) ±3.1**	**18.7** (134%) ±2.3**	**2.9** (480%) ±1.6**	**3.43** (174%) ±0.63**	**25.29** (383%) ±2.93**	**14.09 **(1300%) ±2.69**	NT	NT	NT
259	NT	NT	NT	NT	NT	NT	12/56 (21 %)	4/46 (9 %)	16/56 (29 %)
619-714	**22.3** (185%) ±9.4**	**35.7** (346%) ±10.1**	**11.4** (2180 %) ±3.4**	**9.03** (622%) ±2.54**	**35.41** (576%) ±2.49**	**20.61**(1947%) ±3.57**	**25/59 (42 %) ****	**15/44 (34 %) ****	**40/59 (68 %) ****

NT: Not tested * Significantly different than control ($p<0.05$) ** Significantly different than control ($p<0.01$) SD: Standard deviation

Note 1: The area occupied by peroxisomes was expressed in relation to cytoplasmic region as %. Size classes 1-5 (<0.1 - >0.75 μm^2).

Note 2: Significance of pairwise comparison with control (Fisher's Exact Test) denoted at Dose level. Tumor bearing animals/animal at risk (i.e., the animals, which died prior to the week of the first tumor occurrence for each tumor type, are removed from the animals at risk).

a) 4-week treatment at 42, 223, and 619 mg/kg/day

b) 4 week treatment at 37, 180, and 709 mg/kg/day

c) 1 week treatment at 40, 227, and 714 mg/kg/day

d) 18 month study. Overall mean doses were 119, 259, and 655 mg/kg/day.

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Table 2. Summary of Liver Toxicity and Tumor Induction in Male Mice Administered Acifluorfen

Dose (mg/kg/day) as active ingredient	Subchronic study (a) (MRID 00252826)		Cell Proliferation Study (b) (MRID 45803601)					18-month carcinogenicity study in mice (MRID 00122732)		
	Fatty infiltration of liver	hypertrophy, mitotic activity and increased levels of SGPT and ALP	Hypertrophy	Single cell necrosis and apoptotic cells	Body weight, g (% of controls)	Absolute Liver weight, g (% of controls)	Relative Liver weight (%of controls)	Body weight, % of controls	Liver weight, g (% of controls)	Adenomas/ Carcinomas Combined
0					30.6	1.51	4.94		2.03	9/58 (16 %)
40-48	NF	NF	NF	NF	30.5 (99.7%)	**1.63 (108%)****	**5.34 (108)****	NT	NT	NT
119	NT	NT	NT	NT	NT	NT	NT	**90****	2.41 (119%)	**21/60 (35 %) ***
188	+++ (d)	NF	NT	NT	NT	NT	NT	NT	NT	NT
227	NT	NT	NF	NF	31.3 (102%)	**2.13 (141%)****	**6.80 (137%) ****	NT	NT	NT
259	NT	NT	NT	NT	NT	NT	NT	**87****	**2.95 ** (145%)**	16/56 (29 %)
375	+++	+++	NT	NT	NT	NT	NT	NT	NT	NT
655-750	+++	+++	++ +	+++	**27.3 (89%) ****	**1.90 (125%)****	**6.94 (140%) ****	**75****	**3.75 ** (185%)**	**40/59 (68 %) ****

NT: Not tested NF: Not found * Significantly different than control ($p<0.05$) ** Significantly different than control ($p<0.01$)

a) Treated for 90-days at 3, 12, 48, 188, 375, and 750 mg/kg/day.

b) Treated for 2 weeks at 40, 227, and 714 mg/kg/day

c) 18 month study. Overall mean doses were 119, 259, and 655 mg/kg/day. d) +++ means positive responses.

Table 3. Summary of Peroxisomal Effects and Liver Tumor Induction in Female Mice Administered Acifluorfen

Dose (mg/kg/day) as active ingredient	Mean Number of Peroxisome according to size classification (relative to controls, %) MRID 45693401 (a)				Induction of Peroxisomal Enzyme Activities; Palmitoyl CoA oxidase MRID 45793901 (b)	Cell proliferation; BrdU labeling in the liver (S-phase response) MRID 45803601 (c)	Tumor incidence (% incidence) 18-month carcinogenicity study in mice MRID 00122732 (d) (Note 2)		
	1 (<0.1 μm^2)	2 (<0.3 μm^2)	3 (<0.5 μm^2)	Total area of peroxisomes (note 1)			Adenomas	Carcinomas	Adenomas/ Carcinomas Combined
0	9.5 ±2.8 (SD)	8.2 ±0.7	0.4 ±0.3	1.27 ±0.13	5.65 ±0.94	1.02 ±0.24	1/55 (1 %)	0/45 (0 %)	1/55 (2 %)
54-55					5.81 (+3%) ±0.54	**1.31* (28%)** ±0.31	NT	NT	NT
64	**13.2* (39%)** ±1.4	7.5 ±0.8	0.2 ±0.2	1.25 ±0.18					
143	NT	NT	NT	NT	NT	NT	5 /59 (5 %)	1/47 (2 %)	6/59 (10 %)
255-313	12.1 (27%) ±2.1	**15.9** (94%) ±1.5**	**2.2** (450%) ±1.0**	**2.81** (121%) ±0.37**	**17.84** (+216%) ±1.79**	**3.68** (259%) ±0.70**	4/57 (4 %)	1/44 (2 %)	5/57 (9 %)
711	NT	NT	NT	NT	NT	NT	**19/58 (19 %) ** **	**5/46 (11 %) ***	**24/58 (41 %) ** **
845-933	14.6 (54%) ±5.9	**35.7** (335%) ±11.4**	**14.0** (3400%) ±3.9**	**10.6** (735%) ±2.95**	**45.57** (+707%) ±3.65**	**6.19** (505%) ±1.37**	NT	NT	NT

NT: Not tested * Significantly different than control ($p<0.05$) ** Significantly different than control ($p<0.01$) SD: Standard deviation

Note 1: The area occupied by peroxisomes was expressed in relation to cytoplasmic region as %. Size classes 1-5 (<0.1 - >0.75 μm^2).

Note 2: Significance of pairwise comparison with control (Fisher's Exact Test) denoted at Dose level. Tumor bearing animals/animal at risk (i.e., the animals, which died prior to the week of the first tumor occurrence for each tumor type, are removed from the animals at risk).

a) 4 week treatment at 64, 296, and 892 mg/kg/day. b) 4 week treatment at 55, 255, and 933 mg/kg/day

c) 1 week treatment at 54, 287, and 845 mg/kg/day d) 18 month study. Overall mean doses were143, 313, and 711 mg/kg/day.

Table 4. Summary of Liver Toxicity and Tumor Induction in Female Mice Administered Acifluorfen

Dose (mg/kg/d) as active ingredient	Subchronic study (a) MRID 00252826		Cell Proliferation Study (b) (MRID 45803601)					18-month carcinogenicity study in mice [c] (MRID 00122732)		
	Fatty infiltration of liver	hypertrophy, mitotic activity and increased levels of SGPT and ALP	Hypertrophy	Single cell necrosis and apoptotic cells	Body weight, g (% of controls)	Absolute Liver weight, g (% of controls)	Relative Liver weight (%of controls)	Body weight, % of controls	Liver weight, g (% of controls)	Adenomas / Carcinomas Combined
0					25.6	1.3	5.07		1.70	1/55 (2%)
48-54	NF	NF	NF	NF	25.6	1.31	5.14 (101%)	NT	NT	NT
143	NT	NT	NT	NT	NT	NT	NT	89**	1.59 (96%)	6/59 (10 %)
188	+++[d]	NF	NT	NT	NT	NT	NT	NT	NT	NT
287-313	NT	NT	NF	NF	25.5	1.62 (125%) **	6.35 (125%)**	78**	1.90 (112%) *	5/57 (9 %)
375	+++	+++	NT	NT	NT	NT	NT	NT	NT	NT
711-845	+++	+++	+++	NF	24.8 (97%)	2.19 (168%) **	8.81 (174%) **	66**	2.32 (136%) **	24/58 (41 %) **

NT: Not tested NF: Not found * Significantly different than control ($p<0.05$) ** Significantly different than control ($p<0.01$)

a) Treated for 90-days at 3, 12, 48, 188, 375, and 750 mg/kg/day.

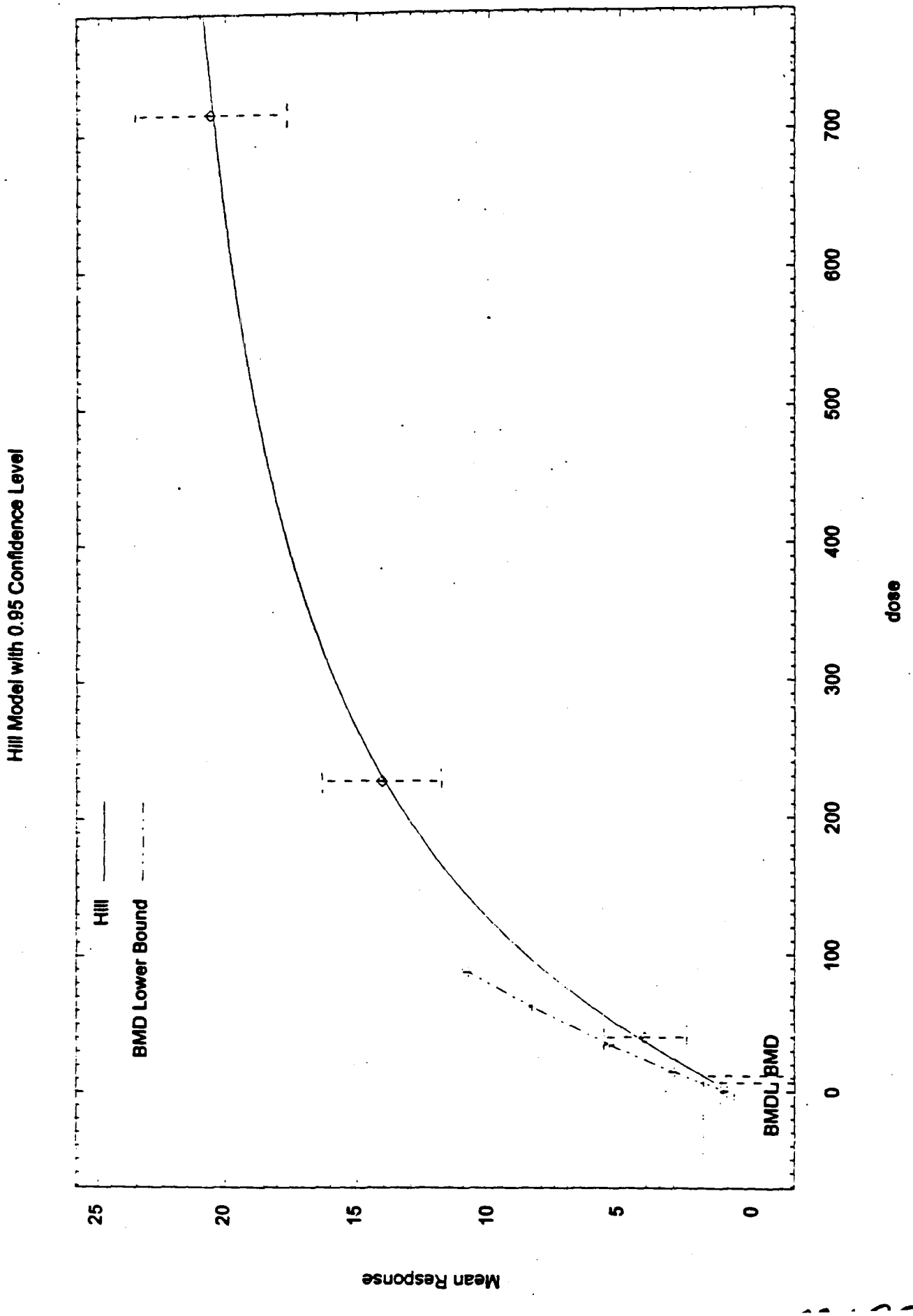
b) Treated for 2 weeks at 54, 287, and 845 mg/kg/day

c) 18 month study. Overall mean doses were143, 313, and 711 mg/kg/day.

d) +++ means positive responses.

Attachment 1: Benchmark Dose Modeling
(not available electronically)

11-25


Hill Model with 0.95 Confidence Level
Hill
BMD Lower Bound
BMDL
BMD
Mean Response
25
20
15
10
5
0
0
100
200
300
400
500
600
700
dose
14:51 05/22 2003

```
 ================================================================================
===
          Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23
$
          Input Data File: C:\KRAFFAEL\DER\KCR\BENCHMARKDOSE\BURNA
MDATA2.(d)
          Gnuplot Plotting File:  C:\KRAFFAEL\DER\KCR\BENCHMARKDOS
E\BURNAMDATA2.plt
                                                          Thu May 22
 14:51:58 2003
 ================================================================================
===

 BMDS MODEL RUN
--------------------------------------------------------------------------------
---

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = COLUMN1
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = alpha * mean(i) ^ rho

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                  Default Initial Parameter Values
                        alpha =     0.145577
                          rho =      1.56187
                    intercept =         1.01
                            v =         19.6
                            n =      0.42722
```


1025

k = 288.214

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	intercept	v	n	k
alpha	1	-0.9	-0.38	0.017	0.082	-0.019
rho	-0.9	1	0.33	-0.034	-0.085	0.023
intercept	-0.38	0.33	1	-0.06	0.053	0.0062
v	0.017	-0.034	-0.06	1	-0.87	0.96
n	0.082	-0.085	0.053	-0.87	1	-0.91
k	-0.019	0.023	0.0062	0.96	-0.91	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.172749	0.101697
rho	1.48623	0.288825
intercept	0.949352	0.138176
v	23.9589	4.57021
n	1.13855	0.216714
k	194.922	86.5682

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev

Chi^2 Res.					
0	8	1.01	0.3	0.949	0.4
0.152					
40	8	4.07	1.86	4.34	1.24
-0.217					
227	8	14.1	2.69	14	2.95
0.0424					
714	8	20.6	3.57	20.5	3.92
0.0386					

Model Descriptions for likelihoods calculated

Model A1: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma^2$

Model A2: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = Sigma(i)^2$

Model A3: $Yij = Mu(i) + e(ij)$
$Var\{e(ij)\} = alpha*(Mu(i))^{rho}$

Model R: $Yi = Mu + e(i)$
$Var\{e(i)\} = Sigma^2$

Warning: Likelihood for fitted model larger than the Likelihood for model A3.

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-42.215615	5	94.431230
A2	-27.293182	8	70.586363
A3	-29.939462	6	71.878924
fitted	-29.939462	6	71.878924
R	-83.654930	2	171.309860

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?

(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	112.723	6	<.0001
Test 2	29.8449	3	<.0001
Test 3	5.29256	2	0.07091
Test 4	-1.64277e-011	0	NA

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .05. A non-homogeneous varian ce
model appears to be appropriate

The p-value for Test 3 is greater than .05. The modeled variance appears
to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0. T he Chi-Square
test for fit is not valid

Benchmark Dose Computation
Specified effect = 1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 11.8537

BMDL = 6.59776

13544

R108195

Chemical:	**Acifluorfen**
PC Code:	**114401**
HED File Code	**61100 SRRD SDTM**
Memo Date:	**07/09/2003**
File ID:	**TX0052014**
Accession Number:	**412-05-0097**

HED Records Reference Center
06/07/2005